FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **002.00120**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **Not Yet Known** 09/581651

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB98/03766	**15 December 1998**	**16 December 1997**

TITLE OF INVENTION
POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

APPLICANT(S) FOR DO/EO/US
Schor, Seth Lawrence
Schor, Ana Maria

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Communication (1 pg)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/581651 Not Yet Known	PCT/GB98/03766	002.00120

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$970.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$840.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$690.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$670.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$96.00**
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	98 - 20 =	78	x $18.00	**$1,404.00**
Independent claims	24 - 3 =	21	x $78.00	**$1,638.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$260.00**
	TOTAL OF ABOVE CALCULATIONS =			**$4,272.00**
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☐			**$0.00**
	SUBTOTAL =			**$4,272.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**
	TOTAL NATIONAL FEE =			**$4,272.00**
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐			**$0.00**
	TOTAL FEES ENCLOSED =			**$4,272.00**
				Amount to be: refunded $
				charged $

☒ A check in the amount of **$4,272.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0772** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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34,103
REGISTRATION NUMBER

15 June 2000
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Schor and Schor
Serial No.:	To be assigned (U.S. National Stage of PCT/GB98/03766)
Filed:	Herewith
For:	POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231
Box PCT

Dear Sir:

Please amend the subject application as follows (amendments refer to the claims as published; please disregard the amendments made during preliminary examination before the European Patent Office):

In the Claims:

Please amend claims 3-8, 13, 24, 35, 39, 44, 47, 49, and 51-58 as follows:

3. (Amended) A polynucleotide according to Claim 1 [or 2], which contains no introns.

4. (Amended) A polynucleotide according to [any one of the preceding claims] Claim 1, comprising the polynucleotide whose sequence is shown in Figure 1.

5. (Amended) A polynucleotide according to [any one of the preceding claims] Claim 1, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.

6. (Amended) A polynucleotide according to [any one of the preceding claims] Claim 1, encoding a polynucleotide which has migration stimulating factor activity.

7. (Amended) A replicable vector comprising a polynucleotide as defined in [any one of Claims 1 to 6] Claim 1.

8. (Amended) A host cell comprising a recombinant polynucleotide as defined in Claim 1 or a replicable vector comprising the polynucleotide [as defined in any one of Claims 1 to 7].

13. (Amended) A polypeptide according to Claim 10 [any one of Claims 10 to 12], which has migration stimulating factor activity.

24. (Amended) An antibody according to any one of Claims 14 to [24] 17 and 19 to 22 which is a monoclonal antibody.

35. (Amended) A polynucleotide according to any one of Claims 31 to 33 [34], wherein the polynucleotide which encodes fibronectin or the polynucleotide which encodes the polypeptide as said or a natural variant thereof is a mRNA or a cDNA.

39. (Amended) A method according to any one of Claims 36 to 38, wherein the reagent which can distinguish said polypeptide from fibronectin is an antibody according to any one of Claims 14 to 17 [18].

44. (Amended) A method according to any one of Claims 36 to 38 and 40 to 42 [43], wherein the cancer is breast cancer.

47. (Amended) A method of modulating cell migration the method comprising administering an effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13] to the site where modulation of cell migration is required.

49. (Amended) A method according to Claim 47 [or 48], wherein the site is in a mammalian body.

51. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], in the manufacture of an agent for modulating cell migration.

52. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], for modulating cell migration.

53. (Amended) A method of healing a wound the method comprising administering to the locality of the wound an

effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13].

54. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], in the manufacture of a medicament for healing wounds.

55. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], for healing wounds.

56. (Amended) A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 and 12 [to 13] and a pharmaceutically acceptable carrier.

57. (Amended) A polypeptide according to any one of Claims 10 and 12 [to 13] for use in medicine.

58. (Amended) A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13].

Please add new claim 59 as follows:

--59. (New) A polypeptide according to Claim 12, which has migration stimulating factor activity.--

REMARKS

Claims 1-58 are presented for examination in the subject application, as published. By this preliminary amendment, claims 3-8, 13, 24, 35, 39, 44, 47, 49, and 51-58 have been amended to adjust the dependencies of the claims. New claim 59 has been added.

Respectfully submitted,

Dated: 15 June 2000

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POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

The present invention relates to polypeptides, polynucleotides and uses thereof and in particular to migration stimulating factor (MSF).

MSF has been described previously in the following papers. Schor *et al* (1988) *J. Cell Sci.* **90**: 391-399 shows that foetal and cancer patient fibroblasts produce an autocrine migration stimulating factor not made by normal adult cells. Schor *et al* (1988) *J. Cell Sci.* **90**: 401-407, shows that fibroblasts from cancer patients display a mixture of both foetal and adult phenotypic characteristics. Schor *et al* (1989) *In Vitro* **25**: 737-746 describes a mechanism of action of the migration stimulating factor (MSF) produced by fetal and cancer patient fibroblasts and its effect on hyaluronic acid synthesis. Grey *et al* (1989) *Proc. Natl. Acad. Sci. (USA)* **86**: 2438-2442 describes the purification of the migration stimulating factor produced by fetal and cancer patient fibroblasts but no amino acid sequence information is given. It is suggested that MSF has a molecular weight of 70kDa. Schor & Schor (1990) *Cancer Investig.* **8**: 665-667 describes the characterisation of migration stimulating activity (MSF) and gives evidence for its role in cancer pathogenesis. Picardo *et al* (1991) *Lancet* **337**: 130-133 describes the presence of migration stimulating activity in the serum of breast cancer patients. Ellis *et al* (1992) *J. Cell Sci.* **102**: 447-456 describes the antagonistic effects of transforming growth factor-β1 and MSF on fibroblast migration and hyaluronic acid synthesis and discusses the possible implications for wound healing. Picardo *et al* (1992) *Exp. Mol. Path.* **57**: 8-21, describes the identification of migration stimulating factor in wound fluid. Irwin *et al* (1994) *J. Cell Sci.* **107**: 1333-1346, describes the inter- and intra-site heterogeneity in the expression of fetal-like phenotypic characteristics by gingival fibroblasts

and discusses the potential significance for wound healing. Schor *et al* (1994) *Int J Cancer.* **59**: 25-32 describes the phenotypic heterogeneity in breast fibroblasts and discusses functional anomaly in fibroblasts from histologically normal tissue adjacent to carcinoma. Schor *et al* (1991) In: *Cell Motility Factors* (ed. I Goldberg) pp. 127-146, Birkhauser Press, Basel, describes the heterogeneity amongst fibroblasts in the production of migration stimulating factor (MSF) and discusses implications for cancer pathogenesis. Schor *et al* (1993) In: *Cell behaviour: Adhesion and Motility*. (ed. G. Evans, C. Wigley and R. Warn) Society for Experimental Biology Symposium No. 47, pp. 234-251, describes the potential structural homology of MSF to the gelatin-binding domain of fibronectin its potential mode of action and possible function in health and disease. A small amount of partial amino acid sequence is given, but this sequence is similar to fibronectin and, in fact, is not present in the MSF which has now been cloned and sequenced in the present work (see below). It is suggested that MSF activity isolated from foetal fibroblast conditioned medium consists of three proteins, one with an apparent molecular weight of 119kDa and a double of 43 and 33kDa, and, indeed, it was suggested that MSF could be a proteotytic degradation product of fibronectin. Schor (1995) In: *Epithelial Mesenchymal Interactions in Cancer* (eg. I Goldberg and E Rosen). pp. 273-296. Birkhauser Press, Basel, describes fibroblast subpopulations as accelerators of tumor progression and the potential role of migration stimulating factor. MSF is also discussed in Schor *et al* (1994) In: Mammary Tumorigenesis and Malignant Progression, Kluwer Academic Publishers, Dickson, R. and Lippman, M. (eds).

Thus, MSF is believed to be produced by fibroblasts obtained from a majority of breast cancer patients and is not made by their normal adult

counterparts. It is believed that measuring the levels of MSF, for example, in circulating blood or in serum or in urine, may be useful in identifying patients who have or are susceptible to cancer, or that it may be useful in prognosing the outcome of cancer. MSF producing fibroblasts are present in patients with a number of common epithelial tumours, such as carcinoma of the breast, lung and colon, as well as melanoma, and soft tissue sarcoma.

It is believed that it may be particularly useful to measure the levels of MSF in identifying patients who have or are susceptible to breast cancer, or in prognosing the outcome of breast cancer.

In addition, it is believed that MSF may be useful in wound healing since it is present in a majority of wound fluid samples. The directed migration of fibroblasts into the wound site and the transient increase in hyaluronic acid in granulation tissue during the wound healing response are both consistent with the involvement of MSF. (MSF stimulates the synthesis of a high molecular weight species of hyaluronic acid).

MSF is known to be related to fibronectin since certain antibodies raised to MSF also bind to fibronectin.

Fibronectin is a widely distributed glycoprotein present at high concentrations in most extracellular matrices, in plasma (300 μg/ml), and in other body fluids. Fibronectin is a prominent adhesive protein and mediates various aspects of cellular interactions with extracellular matrices including migration. Its principal functions appear to be in cellular migration during development and wound healing, regulation of cell growth and differentiation, and haemostasis/thrombosis.

Further progress in understanding MSF was hindered by the fact that it has not been clear whether MSF is a degradation or breakdown product of fibronectin, and because MSF appears to be structurally related to fibronectin.

We have now discovered that MSF is not a breakdown product of fibronectin but that it appears, quite unexpectedly, to be a "mini" splice variant of fibronectin. The amino acid sequence of MSF, disclosed for the first time herein, reveals unexpected regions of dissimilarity with fibronectin. This has led to previously unavailable methods of measuring, identifying and localising MSF becoming available. The availability of a polynucleotide encoding MSF, disclosed for the first time herein, makes available methods for producing MSF and useful variants thereof, and makes available new methods of specifically identifying, measuring and localising MSF.

A first aspect of the invention provides a recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y

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Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives.

Figure 2 shows the amino acid sequence encoded by the cDNA insert in pMSF1α which contains the coding sequence for human migration stimulating factor (MSF). Preferably the amino acid sequence is based on that between the most N-terminal methionine and the most C-terminal stop codon (which are marked X). Thus, it is preferred if the polynucleotide encodes a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660 (ie. starting MLRGPG... as marked and encoding ...LGY as marked), or variants of fragments or fusions or derivatives thereof or fusions of said variants or fragments.

Throughout the specification where the term MSF is used, and the context does not indicate otherwise, it includes a polypeptide which has an amino acid sequence given in Figure 2 labelled pMSF1α and, in particular, the amino acid sequence given between positions 19 and 660.

Amino acid residues are given in standard single letter code or standard three letter code throughout the specification.

It will be appreciated that the recombinant polynucleotides of the invention are not polynucleotides which encode fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a polynucleotide which encodes a portion or portions of MSF which are portions that distinguish

MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

The polynucleotide may be DNA or RNA but it is preferred if it is DNA. The polynucleotide may or may not contain introns. It is preferred that it does not contain introns and it is particularly preferred if the polynucleotide is a cDNA.

A polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given in Figure 1. Thus, a polynucleotide of the invention includes the sequence

CAAACTTGGT GGCAACTTGC CTCCCGGTGC GGGCGTCTCT CCCCCACCGT
CTCAACATGC TTAGGGGTCC GGGGCCCGGG CTGCTGCTGC TGGCCGTCCA
GTGCCTGGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC
AGGCTCAGCA AATGGTTCAG CCCCAGTCCC CGGTGGCTGT CAGTCAAAGC
AAGCCCGGTT GTTATGACAA TGGAAAACAC TATCAGATAA ATCAACAGTG
GGAGCGGACC TACCTAGGCA ATGCGTTGGT TTGTACTTGT TATGGAGGAA
GCCGAGGTTT TAACTGCGAG AGTAAACCTG AAGCTGAAGA GACTTGCTTT
GACAAGTACA CTGGGAACAC TTACCGAGTG GGTGACACTT ATGAGCGTCC
TAAAGACTCC ATGATCTGGG ACTGTACCTG CATCGGGGCT GGGCGAGGGA
GAATAAGCTG TACCATCGCA AACCGCTGCC ATGAAGGGGG TCAGTCCTAC
AAGATTGGTG ACACCTGGAG GAGACCACAT GAGACTGGTG GTTACATGTT
AGAGTGTGTG TGTCTTGGTA ATGGAAAAGG AGAATGGACC TGCAAGCCCA
TAGCTGAGAA GTGTTTTGAT CATGCTGCTG GGACTTCCTA TGTGGTCGGA
GAAACGTGGG AGAAGCCCTA CCAAGGCTGG ATGATGGTAG ATTGTACTTG
CCTGGGAGAA GGCAGCGGAC GCATCACTTG CACTTCTAGA AATAGATGCA
ACGATCAGGA CACAAGGACA TCCTATAGAA TTGGAGACAC CTGGAGCAAG
AAGGATAATC GAGGAAACCT GCTCCAGTGC ATCTGCACAG GCAACGGCCG
AGGAGAGTGG AAGTGTGAGA GGCACACCTC TGTGCAGACC ACATCGAGCG
GATCTGGCCC CTTCACCGAT GTTCGTGCAG CTGTTTACCA ACCGCAGCCT
CACCCCCAGC CTCCTCCCTA TGGCCACTGT GTCACAGACA GTGGTGTGGT
CTACTCTGTG GGGATGCAGT GGCTGAAGAC ACAAGGAAAT AAGCAAATGC
TTTGCACGTG CCTGGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC
CAGACTTACG GTGGCAACTC AAATGGAGAG CCATGTGTCT TACCATTCAC
CTACAACGAC AGGACGGACA GCACAACTTC GAATTATGAG CAGGACCAGA
AATACTCTTT CTGCACAGAC CACACTGTTT TGGTTCAGAC TCGAGGAGGA
AATTCCAATG GTGCCTTGTG CCACTTCCCC TTCCTATACA ACAACCACAA
TTACACTGAT TGCACTTCTG AGGGCAGAAG AGACAACATG AAGTGGTGTG
GGACCACACA GAACTATGAT GCCGACCAGA AGTTTGGGTT CTGCCCCATG
GCTGCCCACG AGGAAATCTG CACAACCAAT GAAGGGGTCA TGTACCGCAT
TGGAGATCAG TGGGATAAGC AGCATGACAT GGGTCACATG ATGAGGTGCA
CGTGTGTTGG GAATGGTCGT GGGGAATGGA CATGCATTGC CTACTCGCAG
CTTCGAGATC AGTGCATTGT TGATGACATC ACTTACAATG TGAACGACAC

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ATTCCACAAG CGTCATGAAG AGGGGCACAT GCTGAACTGT ACATGCTTCG
GTCAGGGTCG GGGCAGGTGG AAGTGTGATC CCGTCGACCA ATGCCAGGAT
TCAGAGACTG GGACGTTTTA TCAAATTGGA GATTCATGGG AGAAGTATGT
GCATGGTGTC AGATACCAGT GCTACTGCTA TGGCCGTGGC ATTGGGGAGT
GGCATTGCCA ACCTTTACAG ACCTATCCAA GCTCAAGTGG TCCTGTCGAA
GTATTTATCA CTGAGACTCC GAGTCAGCCC AACTCCCACC CCATCCAGTG
GAATGCACCA CAGCCATCTC ACATTTCCAA GTACATTCTC AGGTGGAGAC
CTGTGAGTAT CCCACCCAGA AACCTTGGAT ACTGAGTCTC CTAATCTTAT
CAATTCTGAT GGTTTCTTTT TTTCCCAGCT TTTGAGCCAA CAACTCTGAT
TAACTATTCC TATAGCATTT ACTATATTTG TTTAGTGAAC AAACAATATG
TGGTCAATTA AATTGACTTG TAGACTGAAA AAAAAAAAAA AAAAAAA
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It is particularly preferred if the polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given between positions 57 and 1982 in Figure 1 since this is believed to be the coding sequence for human MSF.

The invention includes a polynucleotide comprising a fragment of the recombinant polynucleotide of the first aspect of the invention. Preferably, the polynucleotide comprises a fragment which is at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length. Such polynucleotides are useful as PCR primers.

A "variation" of the polynucleotide includes one which is (i) usable to produce a protein or a fragment thereof which is in turn usable to prepare antibodies which specifically bind to the protein encoded by the said polynucleotide or (ii) an antisense sequence corresponding to the polynucleotide or to a variation of type (i) as just defined. For example, different codons can be substituted which code for the same amino acid(s) as the original codons. Alternatively, the substitute codons may code for a different amino acid that will not affect the activity or immunogenicity of the protein or which may improve or otherwise modulate its activity or immunogenicity. For example, site-directed mutagenesis or other

techniques can be employed to create single or multiple mutations, such as replacements, insertions, deletions, and transpositions, as described in Botstein and Shortle, "Strategies and Applications of *In Vitro* Mutagenesis," *Science*, **229:** 193-210 (1985), which is incorporated herein by reference. Since such modified polynucleotides can be obtained by the application of known techniques to the teachings contained herein, such modified polynucleotides are within the scope of the claimed invention.

Moreover, it will be recognised by those skilled in the art that the polynucleotide sequence (or fragments thereof) of the invention can be used to obtain other polynucleotide sequences that hybridise with it under conditions of high stringency. Such polynucleotides includes any genomic DNA. Accordingly, the polynucleotide of the invention includes polynucleotide that shows at least 55 per cent, preferably 60 per cent, and more preferably at least 70 per cent and most preferably at least 90 per cent homology with the polynucleotide identified in the method of the invention, provided that such homologous polynucleotide encodes a polypeptide which is usable in at least some of the methods described below or is otherwise useful. It is particularly preferred that in this embodiment, the polynucleotide is one which encodes a polypeptide containing a portion or portions that distinguish MSF from fibronectin.

It is believed that MSF is found in mammals other than human. The present invention therefore includes polynucleotides which encode MSF from other mammalian species including rat, mouse, cow, pig, sheep, rabbit and so on.

Per cent homology can be determined by, for example, the GAP program of the University of Wisconsin Genetic Computer Group.

DNA-DNA, DNA-RNA and RNA-RNA hybridisation may be performed in aqueous solution containing between 0.1XSSC and 6XSSC and at temperatures of between 55°C and 70°C. It is well known in the art that the higher the temperature or the lower the SSC concentration the more stringent the hybridisation conditions. By "high stringency" we mean 2XSSC and 65°C. 1XSSC is 0.15M NaCl/0.015M sodium citrate. Polynucleotides which hybridise at high stringency are included within the scope of the claimed invention.

"Variations" of the polynucleotide also include polynucleotide in which relatively short stretches (for example 20 to 50 nucleotides) have a high degree of homology (at least 80% and preferably at least 90 or 95%) with equivalent stretches of the polynucleotide of the invention even though the overall homology between the two polynucleotides may be much less. This is because important active or binding sites may be shared even when the general architecture of the protein is different.

By "variants" of the polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter the activity of the said MSF.

Variants and variations of the polynucleotide and polypeptide include natural variants, including allelic variants and naturally-occurring mutant forms.

MSF may be assessed in bioassays based on its stimulation of adult skin fibroblast migration, for example, as is described in Picardo *et al* (1991) *The Lancet* **337**, 130-133. Specificity for MSF may be inferred by neutralisation of migration stimulating activity by anti-MSF polyclonal antibodies (as herein disclosed). MSF may also be assayed using immunological techniques such as ELISA and the like.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis well known in the art.

Preferably, the variant or variation of the polynucleotide encodes a MSF that has at least 30%, preferably at least 50% and more preferably at least 70% of the activity of a natural MSF, under the same assay conditions.

By "fragment of MSF" we include any fragment which retains activity or which is useful in some other way, for example, for use in raising antibodies or in a binding assay, but which is not a fragment of MSF which could also be a fragment of fibronectin.

By "fusion of MSF" we include said MSF fused to any other polypeptide. For example, the said protein kinase may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of MSF, or it may be fused to some other polypeptide which imparts some desirable characteristics on the MSF fusion. Fusions to any

variant, fragment or derivative of MSF are also included in the scope of the invention.

A further aspect of the invention provides a replicable vector comprising a recombinant polynucleotide encoding MSF, or a variant, fragment, derivative or fusion of MSF or a fusion of said variant, fragment or derivative.

A variety of methods have been developed to operably link polynucleotides, especially DNA, to vectors for example *via* complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are

DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the DNA encoding the polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491. This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art.

In this method the DNA to be enzymatically amplified is flanked by two specific primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

The DNA (or in the case of retroviral vectors, RNA) is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the DNA encoding the polypeptide constituting the compound of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the

polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance.

Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

The vectors typically include a prokaryotic replicon, such as the ColE1 *ori*, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical prokaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and p*Trc*99A and pKK223-3 available from Pharmacia, Piscataway, NJ, USA.

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *HIS3, TRP1, LEU2* and *URA3*. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Other vectors and expression systems are well known in the art for use with a variety of host cells.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research

Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic and kidney cell lines. Yeast host cells include YPH499, YPH500 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 and 293 cells which are human embryonic kidney cells. Preferred insect cells are Sf9 cells which can be transfected with baculovirus expression vectors.

Transformation of appropriate cell hosts with a DNA construct of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen *et al* (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110 and Sambrook *et al* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Transformation of yeast cells is described in Sherman *et al* (1986) *Methods In Yeast Genetics, A Laboratory Manual*, Cold Spring Harbor, NY. The method of Beggs (1978) *Nature* **275**, 104-109 is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA.

Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

For example, many bacterial species may be transformed by the methods described in Luchansky *et al* (1988) *Mol. Microbiol.* **2**, 637-646 incorporated herein by reference. The greatest number of transformants is consistently recovered following electroporation of the DNA-cell mixture suspended in 2.5X PEB using 6250V per cm at 25μFD.

Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* **194**, 182.

Successfully transformed cells, ie cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* **98**, 503 or Berent *et al* (1985) *Biotech.* **3**, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant DNA, successful transformation can be confirmed by well known immunological methods when the recombinant DNA is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity.

Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium.

A further aspect of the invention provides a method of making MSF or a variant, derivative, fragment or fusion thereof or a fusion of a variant, fragment or derivative, the method comprising culturing a host cell comprising a recombinant polynucleotide or a replicable vector which encodes said MSF or variant or fragment or derivative or fusion, and isolating said MSF or a variant, derivative, fragment or fusion thereof of a fusion or a variant, fragment or derivative from said host cell.

Methods of cultivating host cells and isolating recombinant proteins are well known in the art. It will be appreciated that, depending on the host cell, the MSF produced may differ from that which can be isolated from nature. For example, certain host cells, such as yeast or bacterial cells, either do not have, or have different, post-translational modification systems which may result in the production of forms of MSF which may be post-translationally modified in a different why to MSF isolated from nature. It is preferred if the host cell is a non-human host cell; move preferably it is not a mammalian cell.

It is preferred that recombinant MSF is produced in a eukaryotic system, such as an insect cell.

A further aspect of the invention provides MSF or a variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative obtainable by the methods herein disclosed.

A further aspect of the invention provides a polypeptide comprising the amino acid sequence

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

Thus, a polypeptide of the invention includes

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N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
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M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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Preferably, the polypeptide comprises the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

It will be appreciated that the polypeptides of the invention are not fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a portion or portions of MSF which are portions that distinguish MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

Preferably, the polypeptide of the invention is one which has migration stimulating factor activity.

Further aspects of the invention provide antibodies which are selective for MSF (and do not cross react with fibronectin) and antibodies which are selective for fibronectin (and do not cross react with MSF).

By "selective" we include antibodies which bind at least 10-fold more strongly to one polypeptide than to the other (ie MSF *vs* fibronectin); preferably at least 50-fold more strongly and more preferably at least 100-fold more strongly.

Such antibodies may be made by methods well known in the art using the information concerning the differences in amino acid sequence between MSF and fibronectin disclosed herein. In particular, the antibodies may be polyclonal or monoclonal.

Suitable monoclonal antibodies which are reactive as said may be prepared by known techniques, for example those disclosed in "*Monoclonal Antibodies: A manual of techniques*", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", SGR Hurrell (CRC Press, 1982). Polyclonal antibodies may be produced which are polyspecific or monospecific. It is preferred that they are monospecific.

One embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α or natural variants thereof but which epitope is not present in fibronectin.

A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

between positions 19 and 660 or natural variants thereof but which is epitope is not present in fibronectin.

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

ISKYILRWRPVSIPPRNLGY; or

QQWERTYLGNALVCTCYGGSR; or

PCVLPFTYNDRTDSTTSNYEQDQ; or

TDHTVLVQTRGGNSNGALCH; or

VGNGRGEWTCIAYSQLRDQCI

which are found in MSF. The underlined amino acid(s) indicate the difference between MSF and fibronectin.

These peptides contain and flank regions of difference in amino acid sequence between MSF and fibronectin as shown in Figure 2 which are believed to be useful in distinguishing MSF and fibronectin using antibodies.

A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 or natural variants thereof.

A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 between positions 19 and 660 or natural variants thereof.

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof.

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof.

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

QQWERTYLGN<u>V</u>LVCTCYGGSR or

EPCVLPFTYN<u>G</u>RT<u>FYSCTTEGRQDGHLWC</u>STTSNYEQDQ or

CTDHTVLVQT<u>Q</u>GGNSNGALCH or

VGNGRGEWTC<u>Y</u>AYSQLRDQCI or

ISKYILRWRP<u>KNSVGRWKEA</u> or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2. The underlined amino acid(s) indicate the difference between fibronectin and MSF.

These peptides themselves may be useful for raising antibodies, but selective antibodies may be made using smaller fragments of these peptides which contain the region of difference between MSF and fibronectin.

Peptides in which one or more of the amino acid residues are chemically modified, before or after the peptide is synthesised, may be used providing that the function of the peptide, namely the production of specific antibodies *in vivo*, remains substantially unchanged. Such modifications include forming salts with acids or bases, especially physiologically acceptable organic or inorganic acids and bases, forming an ester or amide of a terminal carboxyl group, and attaching amino acid protecting groups such as N-t-butoxycarbonyl. Such modifications may protect the peptide from *in vivo* metabolism. The peptides may be present as single copies or as multiples, for example tandem repeats. Such tandem or multiple repeats may be sufficiently antigenic themselves to obviate the use of a carrier. It may be advantageous for the peptide to be formed as a loop, with the N-terminal and C-terminal ends joined together, or to add one or more Cys residues to an end to increase antigenicity and/or to allow disulphide bonds to be formed. If the peptide is covalently linked to a carrier, preferably a polypeptide, then the arrangement is preferably such that the peptide of the invention forms a loop.

According to current immunological theories, a carrier function should be present in any immunogenic formulation in order to stimulate, or enhance stimulation of, the immune system. It is thought that the best carriers embody (or, together with the antigen, create) a T-cell epitope. The

peptides may be associated, for example by cross-linking, with a separate carrier, such as serum albumins, myoglobins, bacterial toxoids and keyhole limpet haemocyanin. More recently developed carriers which induce T-cell help in the immune response include the hepatitis-B core antigen (also called the nucleocapsid protein), presumed T-cell epitopes such as Thr-Ala-Ser-Gly-Val-Ala-Glu-Thr-Thr-Asn-Cys, beta-galactosidase and the 163-171 peptide of interleukin-1. The latter compound may variously be regarded as a carrier or as an adjuvant or as both. Alternatively, several copies of the same or different peptides of the invention may be cross-linked to one another; in this situation there is no separate carrier as such, but a carrier function may be provided by such cross-linking. Suitable cross-linking agents include those listed as such in the Sigma and Pierce catalogues, for example glutaraldehyde, carbodiimide and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, the latter agent exploiting the -SH group on the C-terminal cysteine residue (if present).

If the peptide is prepared by expression of a suitable nucleotide sequence in a suitable host, then it may be advantageous to express the peptide as a fusion product with a peptide sequence which acts as a carrier. Kabigen's "Ecosec" system is an example of such an arrangement.

The peptide of the invention may be linked to other antigens to provide a dual effect.

A further aspect of the invention provides a method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K

R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin. Suitable peptides are disclosed above.

A still further aspect of the invention provides a method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C

D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF. Suitable peptides are disclosed above.

It will be appreciated that, with the advancements in antibody technology, it may not be necessary to immunise an animal in order to produce an antibody. Synthetic systems, such as phage display libraries, may be used. The use of such systems is included in the methods of the invention.

Before the present invention it was not possible to make use of the differences in amino acid sequence between fibronectin and MSF in order to make antibodies which are useful in distinguishing MSF and fibronectin since it was not known that MSF and fibronectin had significant differences in structure or what those differences were. As is discussed in more detail below, such antibodies are useful in cancer diagnosis. It will also be appreciated that such antibodies which distinguish MSF and fibronectin are also useful research reagents. Suitably, the antibodies of the invention are detectably labelled, for example they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the antibodies are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or they may be linked to an enzyme. Typically, the enzyme is one which can convert a non-coloured (or non-fluorescent) substrate to a coloured (or fluorescent) product. The antibody may be labelled by biotin (or streptavidin) and then detected indirectly using

streptavidin (or biotin) which has been labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or the like or they may be linked to an enzyme of the type described above.

It is particularly preferred if peptides are made, based on the amino acid sequence of MSF and fibronectin, which allow for specific antibodies to be made.

Thus, a further aspect of the invention provides a molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is

```
    N L V A T C L P V R A S L P H R L N
    M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
    R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
    I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
    P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
    W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
    G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
    P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
    V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
    R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
    E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
    Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
    M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
    V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
    V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
    S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
    H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
    C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
    N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
    D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
    Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
    I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
    R P V S I P P R N L G Y
```

or natural variants thereof but not reactive towards fibronectin.

A still further aspect of the invention provides a molecule which is capable of, following immunisation of an animal if appropriate, giving rise to

antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or natural variants thereof.

The molecule is preferably a peptide but may be any molecule which gives rise to the desired antibodies. The molecule, preferably a peptide, is conveniently formulated into an immunological composition using methods well known in the art.

The peptides disclosed above form part of these aspects of the invention.

Peptides may be synthesised by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lu *et al* (1981) *J. Org. Chem.* **46**, 3433 and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is effected using 20% piperidine in N,N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl

esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalising agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N,N-dicyclohexyl-carbodiimide/1-hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50% scavenger mix. Scavengers commonly used are ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesised. Trifluoroacetic acid is removed by evaporation *in vacuo*, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 2QJ, UK. Purification may be effected by any one, or a combination of, techniques

such as size exclusion chromatography, ion-exchange chromatography and (principally) reverse-phase high performance liquid chromatography. Analysis of peptides may be carried out using thin layer chromatography, reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis.

It is now possible to make polynucleotides which can distinguish MSF and fibronectin and such polynucleotides are believed to be useful in the diagnosis and prognosis of cancer.

A further aspect of the invention provides a polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof and a polynucleotide which encodes fibronectin.

A still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant thereof.

A yet still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes the polypeptide which encodes the polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
```

S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

Such polynucleotides can be designed by reference to Figures 1 and 2 and the known sequence of fibronectin (Kornblihtt *et al* (1985) *EMBO J.* **4**, 1755-1759), and may be synthesised by well known methods such as by chemical synthesis or by using specific primers and template, a DNA amplification technique such as the polymerase chain reaction. The polynucleotide may be any polnucleotide, whether DNA or RNA or a synthetic nucleic acid such as a peptide nucleic acid, provided that it can distinguish polynucleotides which encode MSF and fibronectin as said. It is particularly preferred if the polynucleotide is an oligonucleotide which can serve as a hybridisation probe or as a primer for a nucleic acid amplification system. Thus, the polynucleotide of this aspect of the invention may be an oligonucleotide of at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length.

It is particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes MSF but does not hybridise to a mRNA (or cDNA) which encodes fibronectin.

It is also particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes fibronectin but does not hybridise to a

mRNA (or cDNA) which encodes MSF. The nucleotide sequence of MSF cDNA is disclosed herein and the nucleotide sequence of fibronectin is known (for example, see Kornblihtt *et al* (1985) *EMBO J.* **4**, 1755-1759). The skilled person can readily design probes which can distinguish MSF and fibronectin mRNAs and cDNAs based on this information. Differences between MSF and fibronectin include a 45 bp deletion from the first type II fibronectin repeat module in MSF, and the unique tail present in MSF.

Preferably, the polynucleotides of the invention are detectably labelled. For example, they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the polynucleotides are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety or some other suitable detectable moiety. The polynucleotides may be linked to an enzyme, or they may be linked to biotin (or streptaridin) and detected in a similar way as described for antibodies of the invention.

A further aspect of the invention provides a method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y

N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising

detecting in a sample from the patient the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

Preferably, the reagent which can distinguish MSF from fibronectin is an antibody as disclosed herein. The use of antibodies to detect specific polypeptides in samples is well known. For example, they can be used in enzyme-linked immunosorbend assays (ELISA) or they may be used in histopathological analysis. It is believed that the presence of MSF indicates an elevated risk of cancer.

MSF may be conveniently measured in suitable body fluids such as serum or urine, or in extracts of tissue, or in the medium used to culture patient derived cells *in vitro*.

The measurement of MSF is believed to be useful in a number of cancers as discussed above. Antibodies may be used to detect MSF in tissue sections by immunolocalisation. Sub-populations of MSF-producing fibroblasts are present in the normal adult (Irwin *et al* (1994) *J. Cell Science* **107**, 1333-1346; Schor *et al* (1994) pp 277-298 in Mammary Tumorigenesis and Malignant Progression, Dickson, R. and Lippman, M. (eds), 1994, Kluwer Academic Publishers.

It will be appreciated that, as well as the MSF polypeptide being measured using the methods described herein in diagnosis or prognosis or determination of susceptibility to cancer, it may be desirable to detect MSF mRNA in a suitable sample or it may be desirable to detect any changes in the fibronectin gene which are associated with the production of MSF. Mutations in the MSF cDNA or fibronectin gene may be detected using methods well known in the art.

Thus, a further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y

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N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

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N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

Preferably, the reagent which can distinguish the polynucleotide encoding MSF from the polynucleotide encoding fibronectin is a suitable polynucleotide as disclosed herein. Methods of detecting specific nucleic acids in a sample are well known in the art. For example, *in situ* hybridisation methods which detect mRNA may be used, and northern blotting methods may be used. Dot blots, slot blots and Southern blots may also be used.

Thus, it can be seen that the reagents used in the above methods may be used in the manufacture of a reagent for diagnosing cancer.

It will be appreciated that the antibodies of the invention, and the polynucleotides of the invention, which can distinguish MSF and fibronectin (particularly those which recognise MSF or a nucleic acid encoding MSF, but not fibronectin, or a nucleic acid encoding fibronectin) are useful packaged into diagnostic kits containing said antibodies or polynucleotides and other reagents such as means for labelling the said antibodies or polynucleotides.

The invention also includes a number of therapeutic applications, for example chemoprevention and chemotherapy.

Chemoprevention includes the neutralisation of MSF activity and/or the suppression of inappropriate MSF expression in individuals deemed to be at risk of cancer due to inappropriate MSF production. It may also be desirable to administer inhibitors of MSF. Antibodies directed at MSF may act as inhibitors.

Chemotherapy includes the use of anti-MSF antibodies to target coupled cytotoxins to sites of inappropriate MSF production, and the use of MSF inhibitors as mentioned above.

Antibody-targeted cytotoxins are well known in the art and include antibodies linked to a directly cytotoxic moiety such as ricin or a toxic drug; and antibodies linked to an indirectly cytotoxic moiety such as an enzyme which is able to convert a non-toxic prodrug into a toxic drug. In the latter case, the prodrug as well as the antibody-linked enzyme is administered to the patient.

It is useful to measure MSF in wound fluids since this information may be relevant in terms of predicting the efficiency of the subsequent healing process, including the propensity of the scar. The amount of MSF in wound fluids may be measured using, for example, an MSF-selective antibody of the invention.

Inappropriate expression of MSF may be a feature of several pathological conditions characterised by inflammation, such as rheumatoid arthritis. The measurement of MSF in associated body fluid, such as synovial fluid, may be of clinical utility; other pathological conditions of relevance in this context include fibrotic and periodontal disease.

MSF is believed to be involved in the migration of cells, especially fibroblasts any, in particular, the migration of cells may take place within the wound.

Thus, a further aspect of the invention provides a method of modulating cell migration the method comprising administering an effective amount of

a polypeptide of the invention to the site where modulation of cell migration is required.

Typically, the cell whose migration is modulated is a fibroblast. Typically, MSF stimulates the migration of cells such as fibroblasts. Preferably, the site where modulation of cell migration is required is a site within a mammalian body, such as the body of a horse, pig, cow, sheep, cat, dog and the like. Most preferably it is a site within a human body. It is preferred if the site within the body is the site of a wound.

A further aspect of the invention provides a method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide of the invention.

The invention also includes a method of preventing scarring by administering to the locality of the site where scarring is believed to be likely an effective amount of an MSF polypeptide as described herein or a suitable fragment or variant. Preventing or reducing scarring may be part of the wound-healing process. The MSF polypeptide as described herein or a suitable fragment or variant is believed to be useful in preventing or reducing scarring because it reduces hyaluronic acid formation.

It is preferred if the polypeptide administered is a recombinant polypeptide expressed in a eukaryotic host.

The MSF polypeptide may be administered to the site of cell migration or wound healing by any suitable means. Conveniently, the polypeptide is administered topically. It is particularly preferred if the polypeptide is incorporated within an applied wound dressing such as a collagen mesh.

Dressings which are suitable for the incorporation of the polypeptide of the invention are well known in the art and many are commercially available.

Other formulations might involve the incorporation of MSF into an ointment, paste, gel, cream (or equivalent) designed for topical application.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (polypeptide of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient

in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Application of gene therapy techniques may provide a means of controlling MSF expression.

Any suitable amount of the polypeptide of the invention may be administered. By "suitable amount" we mean an amount which gives the desired biological response and that does not lead to any significantly undesirable effects such as toxicity or the like. Small quantities of MSF, for example less than 1 μg, may be effective. It is preferred if superficial wounds, such as those to the skin, are treated by the method of the invention.

The invention will now be described in further detail with reference to the following Figures and Examples wherein:

Figure 1 shows the entire nucleotide sequence of the 2.1kb insert in clone pMSF1α which contains the MSF cDNA. The start and stop codons are underlined.

Figure 2 shows the translation of the cDNA sequence shown in Figure 1 and the alignment of the peptide sequence with that of the gelatin-binding

domain of fibronectin. The start and end of the MSF polypeptide are indicated by vertical bars and arrows.

Figure 3 shows the peptide sequence of MSF (as encoded in the pMSF1α clone) according to its domains. The sequence of pMSF1α is shown grouped according to its domains (cf analysis of fibronectin from Kornblihtt *et al* (1985) *EMBO J.* **4**, 1755-1759). Residues are numbered and have been aligned to give optimal homology by introducing gaps (indicated by ^). Identical residues within a type of homology are indicated by a box (A), and stop codons are designated by asterisks (*). Deleted amino acids are indicated by dashed lines (-), and the IGDS sequence is underlined.

Figure 4 shows a diagrammatic comparison of fibronectin and MSF.

Figure 5 shows a diagrammatic model of MSF showing the positions of the IGD-containing sequences (ie. IGDT, IGDS and IGDQ) within the domains.

Example 1: Cloning and sequence analysis of pMSF1α, a clone encoding MSF

A cDNA library was constructed using mRNA extracted from a human foetal fibroblast cell line, MRC5-SV2, in the vector λZapII.

A primer based on peptide sequence from the gelatin-binding domain (GBD) of fibronectin was used together with a vector primer in the polymerase chain reaction (PCR) to amplify a fragment of 1.2kb. Sequence analysis showed a strong homology to GBD for most of the

fragment. Clear differences included an internal deletion of 45bp, and a 3' unique sequence of 175bp.

The 3' unique sequence was used as a probe for screening the library, using the digoxigenin-labelled system. Positive plaques were picked for further analysis by secondary and tertiary screening, followed by *in vivo* excision of the pBluescript™ phagemid containing the cloned insert.

A plasmid containing an insert of 2.1kb, named pMSF1α, was sequenced by the Sanger-dideoxy method, using a progressive priming approach, and the sequence was assembled into a single contain using the Fragment Assembly System of the Daresbury/Seqnet series of programs.

The entire nucleotide sequence of the 2.1kb fragment is shown in Figure 1.

Translation of this sequence and alignment of its peptide sequence with that of the gelatin-binding domain of fibronectin was achieved using the Fasta program (Daresbury/Seqnet), and is shown in Figure 2.

Figure 3 shows the peptide sequence of pMSF-1α grouped according to its domains.

Other cDNA clones encoding MSF may be readily obtained and sequenced using methods well known in the art and probe derived from the Figure 1 sequence, in particular probes which distinguish MSF from fibronectin.

Example 2: Demonstration of the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast tissue

In situ hybridisation using a riboprobe based on the unique coding region for the unique C-terminus of MSF demonstrates the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast tissue.

Suitable riboprobes contain the entire unique nucleotide sequence of MSF-1α (position 1953-2147), and may include up to 10 bases upstream and contained within the fibronectin sequence (position 1943-2152). This ensures high specificity towards MSF-1α, whilst allowing the use of a probe of longer length. A digoxigenin-labelled riboprobe containing a major portion of the unique sequence (position 1974-2147) is used. This region was selected on the basis of the position of convenient restriction sites.

Example 3: Monoclonal antibodies which are specific to MSF and do not cross-react with fibronectin

Monoclonal antibodies are raised using any of the currently available standard procedures. The immunogen is a synthetic peptide based on the 10 amino acid unique tail of MSF or is based on the peptide sequences:

ISKYILRWRPVSIPPRNLGY; or
QQWERTYLGNALVCTCYGGSR; or
PCVLPFTYNDRTDSTTSNYEQDQ; or
TDHTVLVQTRGGNSNGALCH; or
VGNGRGEWTCIAYSQLRDQCI

Example 4: Genomic PCR and FISH studies

Objective: To obtain information regarding the sequence of the genomic MSF gene regarding (i) its relationship to fibronectin, and (ii) chromosomal location.

Background: The 5′ upstream untranslated sequence of the cloned MSF cDNA is identical to that of fibronectin, thereby strongly suggesting its close relationship to the fibronectin gene (note: such upstream untranslated regions are virtually never identical between two genes as there is no selective pressure. This inference is in apparent conflict with the "uniqueness" of the 3′ end of the MSF cDNA which codes for a 10 amino acid polypeptide and also contains a contiguous untranslated region containing several stop codons).

Methods and Results: Two PCR reactions were established: one at the extreme 5′ untranslated region of fibronectin (FN)/MSF and the other at the extreme 3′ region of MSF which encompassed the 175bp unique sequence. Reactions were carried out using DNA purified using the *Qiagen* Blood kit. Sequence analysis of the resulting amplicon revealed that the 175bp "unique" sequence was contiguous with the fibronectin sequence.

Experiments were then carried out in order to obtain initial data regarding the genomic location of the 3′ unique sequence. This was accomplished by selecting clones from the human PAC library (obtained from HGMP) using the above 2 PCR approach. Secondary and tertiary screening lead

to the identification of on which produced products from *both* PCR reactions. This clone was approximately 70-110 kb in size.

The isolated clone was next subjected to restriction digestion (BamHI and KpnI) and the fragments subcloned into pBluescript and analysed using our 2 PCR approach. Two positive clones were identified: clone B3(2) is 20 kb and can generate both the 5′ and 3′ fragments, thereby indicating that it contains the entire MSF genomic sequence. The other clone, K5(5) is 7 kb and only contains the 3′ unique sequence.

We have used both clones for FISH analysis of the human genome. Our data unambiguously indicate that MSF maps to chromosome 2 region q35. Note: this is within the fibronectin gene, which is located on chromosome 2q34-36.

Conclusions: The FISH analysis clearly indicates that the gene coding for the MSF "unique" sequence is contained within the fibronectin gene. These results indicate that MSF is a novel "mini" splice variant of fibronectin. The genomic fibronectin gene is very large indeed and has still not been fully sequenced. To our knowledge, this is the first report of the unique sequence. The absence of the unique sequence in all previously identified isoforms of fibronectin (which are all in excess of 220 kDa compared to 70 kDa for MSF) indicates that it is spliced out of these molecules.

This information is of relevance for several reasons. Firstly, all previously described splice variants of fibronectin have molecular masses in the region of 225 kDa compared with only 70 kDa of MSF. This small size is totally unexpected and prompts us to refer to MSF as a novel

"mini" splice variant of fibronectin. Secondly, all known splice variants of fibronectin involve the inclusion/deletion of entire type III repeats or variable regions of the IIICS region (all of which occur at a considerable distance downstream of the termination of MSF, which does not contain any known splice site). Finally, as the unique 3'-sequence of MSF was not hitherto identified, it was not possible to predict that MSF was indeed a splice variant of fibronectin until the above data was obtained from genomic DNA.

Example 5: Recombinant MSF expression

Objective: To express recombinant human MSF (rhMSF) in 3T3 cells.

Methods and Results: 3T3 cells were transfected using the Lipfectamine/Plus system (Gibco), according to the manufacturer's instructions. The plasmid used was pcDNA3.1/hisB/lacZ. The insert sequence contained a sequence encoding a *his* tail fused to the human MSF cDNA sequence so that a fusion protein with a *his* tail is expressed. This facilitates purification of the expressed protein. Transfectants were isolated by their selective growth in medium containing 418. One liter of conditioned medium produced by the transfected cells was collected and the fraction containing all the migration stimulating activity obtained by doing a 0-20% ammonium sulphate precipitation. The pellet was resuspended in buffer and the *his*-tagged rhMSF purified by passage through a ProBond column (Invitrogen) column, all done in accordance with manufacturer's instructions. Approximately 250 µg of rhMSF were collected from the starting material. The purified protein resulted in a single band of approximately 70 kDa in SDS PAGE. This protein stimulated the migration of target adult fibroblasts and was active at

concentrations between 1 pg/ml to 10 ng/ml (ie in precise agreement with previously published data regarding the dose-response of MSF purified from fetal fibroblast conditioned medium).

Example 6: Anti-MSF antibody production

Objective: To generate polyclonal antibodies to MSF.

Methods: Rabbits were immunised with a 15-mer synthetic peptide based on the C-terminus of MSF: note, this contains the entire 10 amino acid unique sequence and the contiguous 5 amino sequence of fibronectin. The synthetic peptide was coupled to keyhole limpet haemocyanin (KLH) carrier and used to immunise two rabbits with the following protocol: first injection of 10 mg and second injection of 5 mg three weeks later. Serum was collected six weeks after the first injection and purified IgG shown to recognise the synthetic peptide in both dot and Western blots.

Results: We have used the antibody for both Western blots and immunohistochemistry. The former application has (i) confirmed that rhMSF is recognised by the antibody, and (ii) demonstrated that fetal, but not adult, fibroblasts produce a 70 kDa molecule which is recognised by the antibody and expresses migration stimulating activity when eluted from the PAGE gels.

Polyclonal antibodies were generated against a synthetic peptide incorporating the 10 amino acid "unique" MSF C-terminal sequence. This antibody recognises the unique synthetic peptide (down to 5 ng) and MSF (down to 10 ng) in dot blots; it does not recognise fibronectin or BSA at concentrations up to 4 µg. This antibody has been used to

investigate the tissue distribution of MSF; these experiments show that MSF is present in the stromal compartment of fetal skin and is not detectable in adult skin.

CLAIMS

1. A recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

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N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or variants or fragments or derivatives or fusions thereof or fusions of said variants or fragments or derivatives.

2. A polynucleotide according to Claim 1, encoding a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

3. A polynucleotide according to Claim 1 or 2, which contains no introns.

4. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1.

5. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.

6. A polynucleotide according to any one of the preceding claims, encoding a polypeptide which has migration stimulating factor activity.

7. A replicable vector comprising a polynucleotide as defined in any one of Claims 1 to 6.

8. A host cell comprising a recombinant polynucleotide or a replicable vector as defined in any one of Claims 1 to 7.

9. A method of making a polypeptide having the amino acid sequence

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N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives, the method comprising culturing a host cell as defined in Claim 8 which expresses said variant or fragment

or derivative or fusion and isolating said polypeptide or variant or fragment or derivative or fusion from said host cell culture.

10. A polypeptide comprising the amino acid sequence

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

11. A polypeptide according to Claim 10, comprising the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660, or variants or fragments or fusions thereof or fusions of said variants or fragments.

12. A polypeptide obtainable by the method of Claim 9.

13. A polypeptide according to any one of Claims 10 to 12, which has migration stimulating factor activity.

14. An antibody reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof but not reactive towards fibronectin.

15. An antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.

16. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R

C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof but which epitope is not present in fibronectin.

17. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof but which epitope is not present in fibronectin.

18. An antibody according to any one of Claims 14 to 17, reactive towards a molecule comprising any one of the peptides ISKYILRWRPVSIPPRNLGY or QQWERTYLGNALVCTCYGGSR or EPCVLPFTYNDRTDSTTSNYEQDQ or CTDHTVLVQTRGGNS-NGALCH or VGNGRGEWTCIAYSQLRDQCI .

19. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof.

20. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof.

21. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof.

22. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof.

23. An antibody according to any one of Claims 19 to 22 reactive towards a molecule comprising any one of the peptides

QQWERTYLGNVLVCTCYGGSR or EPCVLPFTYNGRTFYSCTTEG-RQDGHLWCSTTSNYEQDQ or CTDHTVLVQTQGGNSNGALCH or VGNGRGEWTCYAYSQLRDQCI or ISKYILRWRPKNSVGRWKEA or peptides derived from position 648 in fibronectin as shown in Figure 2.

24. An antibody according to any one of Claims 14 to 24 which is a monoclonal antibody.

25. A method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

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N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin.

26. A method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF.

27. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F

I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof but not reactive towards fibronectin.

28. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or natural variants thereof.

29. A molecule according to Claim 27 which is a peptide comprising any one of the sequences

ISKYILRWRPVSIPPRNLGY; or

QQWERTYLGNALVCTCYGGSR; or

PCVLPFTYNDRTDSTTSNYEQDQ; or

TDHTVLVQTRGGNSNGALCH; or

VGNGRGEWTCIAYSQLRDQCI

which are found in MSF.

30. A molecule according to Claim 28, which is a peptide comprising any one of the sequences

QQWERTYLGNVLVCTCYGGSR or

EPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQ or

CTDHTVLVQTQGGNSNGALCH or

VGNGRGEWTCYAYSQLRDQCI or

ISKYILRWRPKNSVGRWKEA or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2.

31. A polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant thereof and a polynucleotide which encodes fibronectin.

32. A polynucleotide which is capable of hybridising to a polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof.

33. A polynucleotide which is capable of hybridising to a polynucleotide which encodes the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

34. A polynucleotide according to any one of Claims 31 to 33, wherein the polynucleotide is an oligonucleotide.

35. A polynucleotide according to any one of Claims 31 to 34, wherein the polynucleotide which encodes fibronectin or the polynucleotide which encodes the polypeptide as said or a natural variant thereof is a mRNA or a cDNA.

36. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

37. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

38. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R

C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

39. A method according to any one of Claims 36 to 38, wherein the reagent which can distinguish said polypeptide from fibronectin is an antibody according to any one of Claims 14 to 18.

40. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed a polynucleotide encoding a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

41. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

42. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

```
N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
```

D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

43. A method according to any one of Claims 40 to 42, wherein the reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin is a polynucleotide according to Claim 31 or 33.

44. A method according to any one of Claims 36 to 43, wherein the cancer is breast cancer.

45. Use of a reagent which can distinguish the polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N
M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y
R I G D T W S K K D N R G N L L Q C I C T G N G R G E W K C
E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P
Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
M L C T C L G N G V S C Q E T A V T Q T Y G G N S N G E P C
V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
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I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

or a natural variant thereof from fibronectin in the manufacture of a reagent for diagnosing cancer.

46. Use of a reagent as defined in Claim 45, as a diagnostic agent.

47. A method of modulating cell migration the method comprising administering an effective amount of a polypeptide according to any one of Claims 10 to 13 to the site where modulation of cell migration is required.

48. A method according to Claim 47, wherein the cell is a fibroblast or an endothelial cell.

49. A method according to Claim 47 or 48, wherein the site is in a mammalian body.

50. A method according to Claim 49, wherein the site is in a human body.

51. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of an agent for modulating cell migration.

52. Use of a polypeptide according to any one of Claims 10 to 13, for modulating cell migration.

53. A method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide according to any one of Claims 10 to 13.

54. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of a medicament for healing wounds.

55. Use of a polypeptide according to any one of Claims 10 to 13, for healing wounds.

56. A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 to 13 and a pharmaceutically acceptable carrier.

57. A polypeptide according to any one of Claims 10 to 13 for use in medicine.

58. A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 to 13.

```
   1  CAAACTTGGT GGCAACTTGC CTCCCGGTGC GGGCGTCTCT CCCCCACCGT
  51  CTCAACATGC TTAGGGGTCC GGGGCCCGGG CTGCTGCTGC TGGCCGTCCA
 101  GTGCCTGGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC
 151  AGGCTCAGCA AATGGTTCAG CCCCAGTCCC CGGTGGCTGT CAGTCAAAGC
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 251  GGAGCGGACC TACCTAGGCA ATGCGTTGGT TTGTACTTGT TATGGAGGAA
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 351  GACAAGTACA CTGGGAACAC TTACCGAGTG GGTGACACTT ATGAGCGTCC
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 851  AGGAGAGTGG AAGTGTGAGA GGCACACCTC TGTGCAGACC ACATCGAGCG
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 951  CACCCCCAGC CTCCTCCCTA TGGCCACTGT GTCACAGACA GTGGTGTGGT
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1051  TTTGCACGTG CCTGGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC
```

Fig. 1 (part 1)

```
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1151  CTACAACGAC AGGACGGACA GCACAACTTC GAATTATGAG CAGGACCAGA
1201  AATACTCTTT CTGCACAGAC CACACTGTTT TGGTTCAGAC TCGAGGAGGA
1251  AATTCCAATG GTGCCTTGTG CCACTTCCCC TTCCTATACA ACAACCACAA
1301  TTACACTGAT TGCACTTCTG AGGGCAGAAG AGACAACATG AAGTGGTGTG
1351  GGACCACACA GAACTATGAT GCCGACCAGA AGTTTGGGTT CTGCCCCATG
1401  GCTGCCCACG AGGAAATCTG CACAACCAAT GAAGGGGTCA TGTACCGCAT
1451  TGGAGATCAG TGGGATAAGC AGCATGACAT GGGTCACATG ATGAGGTGCA
1501  CGTGTGTTGG GAATGGTCGT GGGGAATGGA CATGCATTGC CTACTCGCAG
1551  CTTCGAGATC AGTGCATTGT TGATGACATC ACTTACAATG TGAACGACAC
1601  ATTCCACAAG CGTCATGAAG AGGGGCACAT GCTGAACTGT ACATGCTTCG
1651  GTCAGGGTCG GGGCAGGTGG AAGTGTGATC CCGTCGACCA ATGCCAGGAT
1701  TCAGAGACTG GGACGTTTTA TCAAATTGGA GATTCATGGG AGAAGTATGT
1751  GCATGGTGTC AGATACCAGT GCTACTGCTA TGGCCGTGGC ATTGGGGAGT
1801  GGCATTGCCA ACCTTTACAG ACCTATCCAA GCTCAAGTGG TCCTGTCGAA
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1901  GAATGCACCA CAGCCATCTC ACATTTCCAA GTACATTCTC AGGTGGAGAC
1951  CTGTGAGTAT CCCACCCAGA AACCTTGGAT ACTGAGTCTC CTAATCTTAT
2001  CAATTCTGAT GGTTTCTTTT TTTCCCAGCT TTTGAGCCAA CAACTCTGAT
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```
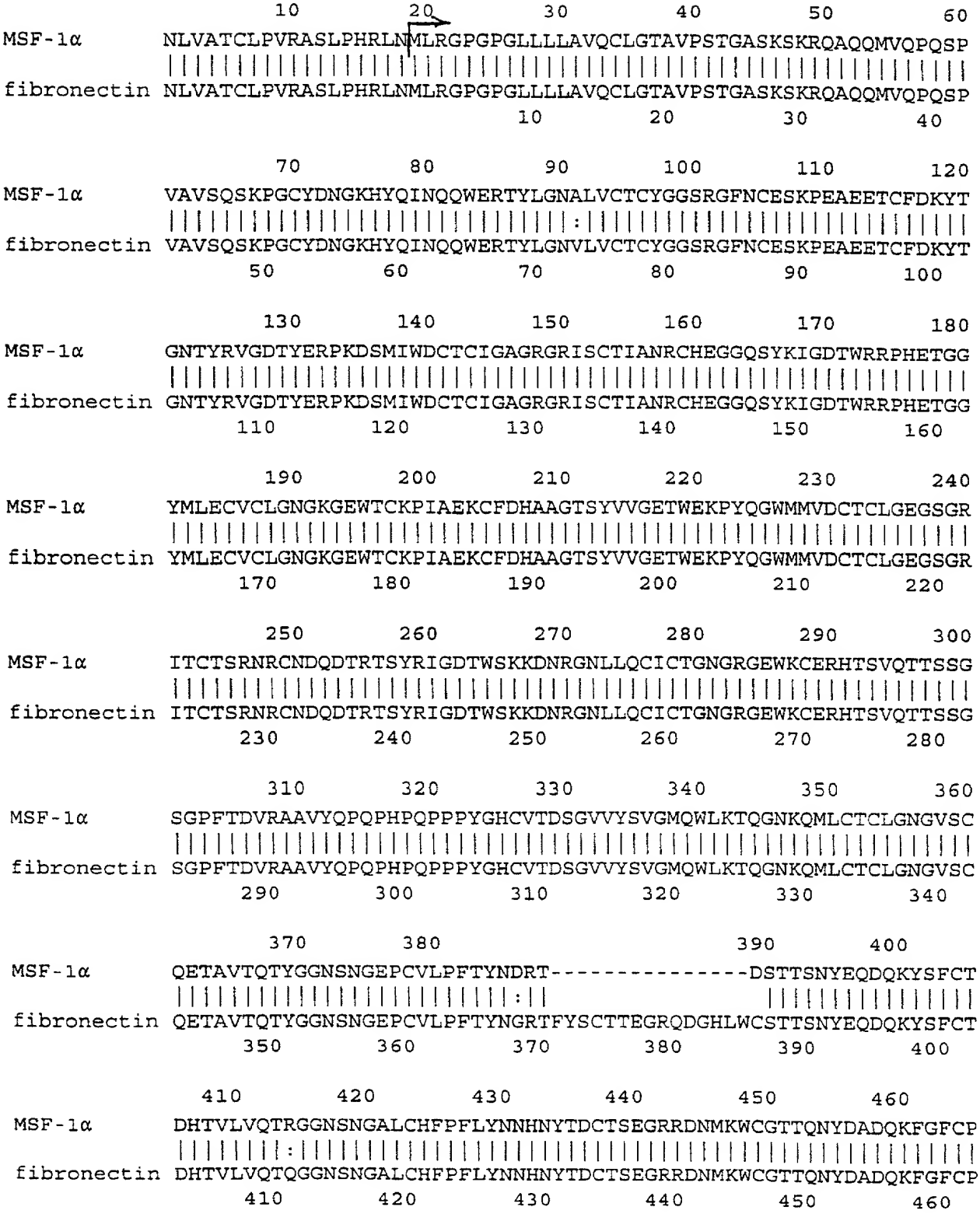

Fig. 1 (part 2)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fibronectin GNTYRVGDTYERPKDSMIWDCTCIGAGRGRISCTIANRCHEGGQSYKIGDTWRRPHETGG
MSF-1α YMLECVCLGNGKGEWTCKPIAEKCFDHAAGTSYVVGETWEKPYQGWMMVDCTCLGEGSGR
fibronectin YMLECVCLGNGKGEWTCKPIAEKCFDHAAGTSYVVGETWEKPYQGWMMVDCTCLGEGSGR
MSF-1α ITCTSRNRCNDQDTRTSYRIGDTWSKKDNRGNLLQCICTGNGRGEWKCERHTSVQTTSSG
fibronectin ITCTSRNRCNDQDTRTSYRIGDTWSKKDNRGNLLQCICTGNGRGEWKCERHTSVQTTSSG
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fibronectin SGPFTDVRAAVYQPQPHPQPPPYGHCVTDSGVVYSVGMQWLKTQGNKQMLCTCLGNGVSC
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fibronectin QETAVTQTYGGNSNGEPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQKYSFCT
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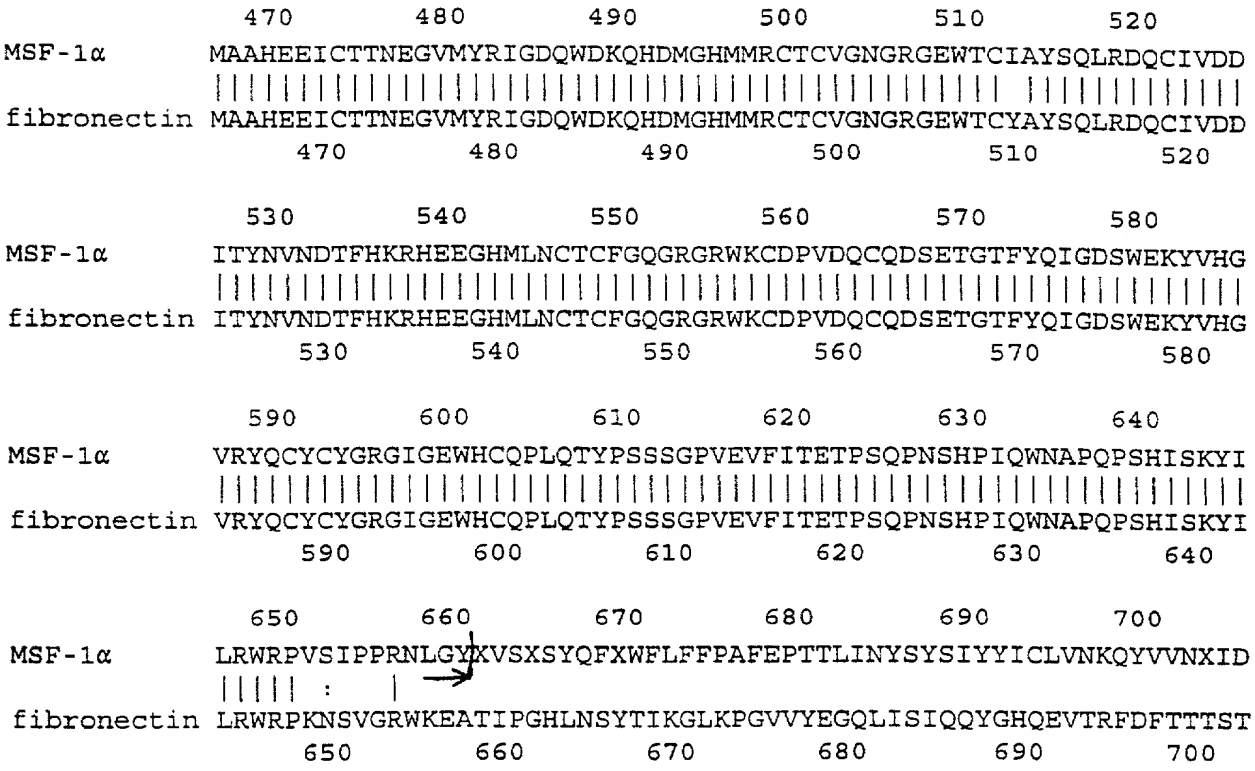
Fig. 2 (part 1)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470 480 490 500 510 520
530 540 550 560 570 580
MSF-1α ITYNVNDTFHKRHEEGHMLNCTCFGQGRGRWKCDPVDQCQDSETGTFYQIGDSWEKYVHG
fibronectin ITYNVNDTFHKRHEEGHMLNCTCFGQGRGRWKCDPVDQCQDSETGTFYQIGDSWEKYVHG
530 540 550 560 570 580
590 600 610 620 630 640
MSF-1α VRYQCYCYGRGIGEWHCQPLQTYPSSSGPVEVFITETPSQPNSHPIQWNAPQPSHISKYI
fibronectin VRYQCYCYGRGIGEWHCQPLQTYPSSSGPVEVFITETPSQPNSHPIQWNAPQPSHISKYI
590 600 610 620 630 640
650 660 670 680 690 700
MSF-1α LRWRPVSIPPRNLGYXVSXSYQFXWFLFFPAFEPTTLINYSYSIYYICLVNKQYVVNXID
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Fig. 2 (part 2)

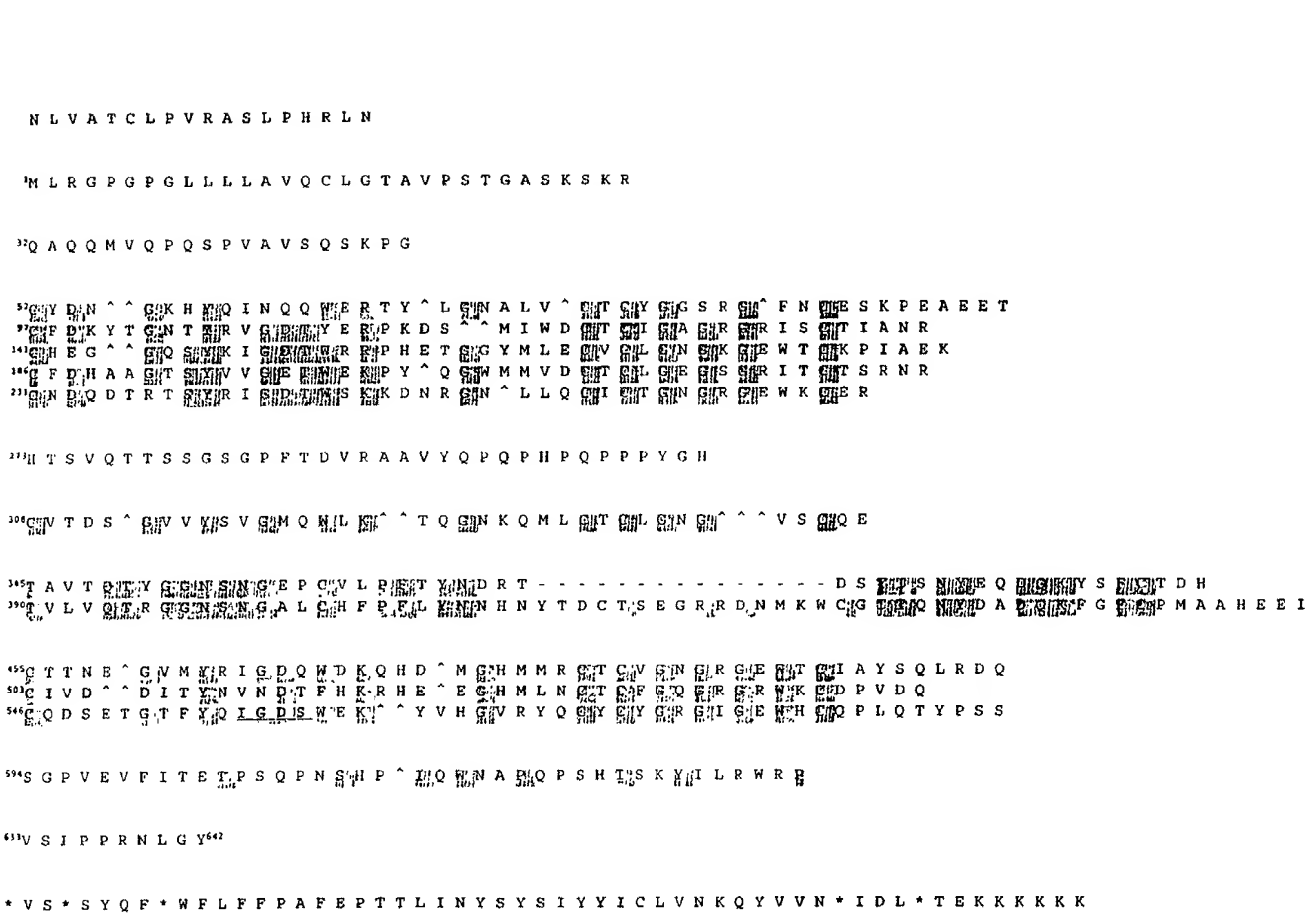

Fig. 3

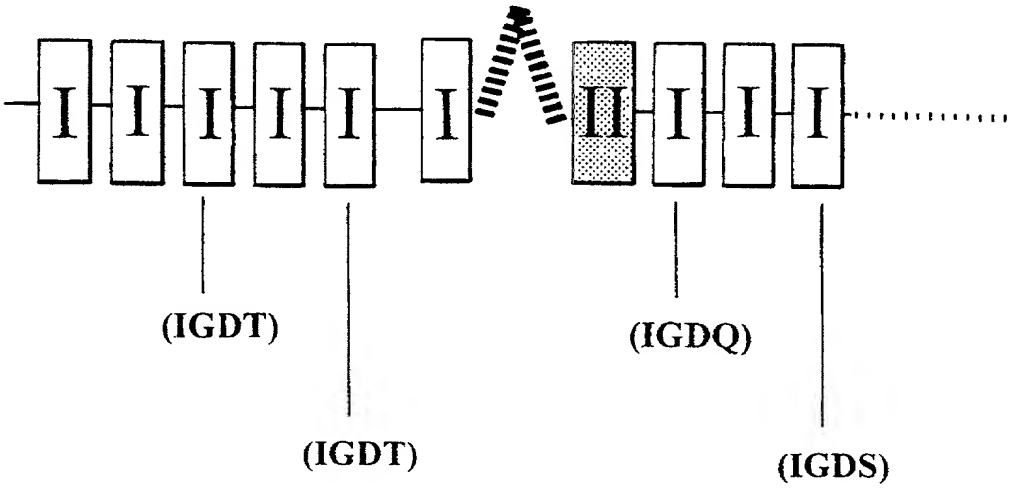

Fig. 4

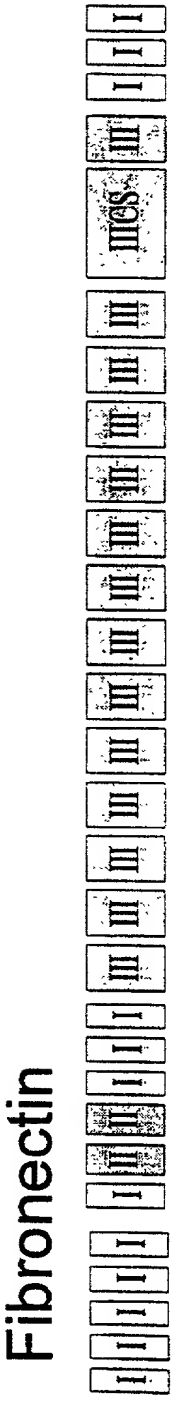

Fig. 5

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	002.00120
First Named Inventor	Schor
COMPLETE IF KNOWN	
Application Number	09/581,651
Filing Date	June 15, 2000
Group Art Unit	Unknown
Examiner Name	Unknown

As a below named inventor, I hereby declare

My residence, post office address, and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 06/15/2000 as United States Application Number or PCT International Application Number 09/581,651 and was amended on (MM/DD/YYYY) _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56

I hereby claim foreign priority benefits under 35 U S C 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
9726539.1	GB	12/16/1997	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S C 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U S C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U S C 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1 56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/GB98/03766	12/15/1998	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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Braman, Susan J.	34,103	Rogalskyj, Peter	38,601
Weyand, Karla M.	40,223		

3

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U S C 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Seth Lawrence	Schor

1-0

Inventor's Signature	Seth Schor					Date	14-08-00
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☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

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Inventor's Signature						Date	
Residence City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						Date	
Residence City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
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